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Epidemiology-based Analysis of Characteristics of Dual Infection of Tuberculosis /Acquired Immune Deficiency Syndrome (AIDS) and Drug Resistance Mechanism of Related Genes

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ABSTRACT

This research was to explore the population characteristics and drug-resistant gene mutations of tuberculosis (TB) and human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) dual infection population, and to provide a reference for clinical screening and prevention of TB/HIV dual infection. TB patients and HIV-infected/AIDS patients registered in Fuzhou Center for Disease Control and Prevention were selected as research subjects. The population characteristics of TB/HIV dual infection and mutation of drug-resistant genes were discussed. It was found that TB patients aged 20-40 years had the highest HIV infection rate, followed by those aged over 40 years. The rate of HIV infection in smear-negative TB patients was higher than that in smear-positive TB patients. HIV/AIDS patients aged 20-40 had the highest TB infection rate. In addition, men had higher rates of HIV than women, and married people had lower rates of HIV than single people. Mycobacterium tuberculosis (MTB) had the highest resistance to isoniazid (42.86%), followed by ofloxacin (34.82%), streptomycin (33.81%), and rifampicin (32.15%). Among the 113 cases of multi-drug resistant strains, 82 cases had mutations in the rpoB gene, with a gene mutation rate of 55.75%. The mutations ranged from codon 511 to codon 569. A total of 31 cases had mutations in the katG/inhA gene. Of which, there were 17 cases of katG single gene mutation, 9 cases of inhA single gene mutation, and 5 cases of combined katG and inhA gene mutation. It was suggested that it was necessary to carry out key TB/HIV two-way screening for TB patients older than 40 years old/smear-negative and male, single, and HIV-infected/AIDS patients aged 20-40. The resistance of MTB to antiTB drugs in this area was generally high, and the drug resistance of retreated patients was significantly higher than that of newly treated patients. Among the resistance genes, the rpoB gene had the highest mutation frequency, followed by the katG gene and inhA gene.

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Introduction

Tuberculosis (TB) and human immunodeficiency virus (HIV) are two chronic infectious diseases worldwide. HIV is also called acquired immune deficiency syndrome (AIDS), which seriously threatens human health and safety (1,2). The occurrence and development of TB and HIV are related to Mycobacterium tuberculosis (MTB), HIV infection, as well as human behavior and social factors. TB and HIV are not only simple global health problems, but are also directly related to world politics, economy, and human life (3-5). Statistics from the Ministry of Health showed that the number of people infected with HIV worldwide was estimated to be more than 33.4 million, and about one-third of the world's 6 billion people were infected with TB bacteria. TB not only directly threatens human health but also acts as an accomplice of HIV (6). When a person infected with HIV is infected with TB bacteria, the incidence of TB is 30 times higher than that of HIV-negative persons (7). After the mid-1990s, many patients with dual infection of MTB and human immunodeficiency virus appeared in all countries in the world. Therefore, the normalized prevention and control of TB combined with HIV dual infection (TB/HIV) must be carried out seriously (8,9).

Multi-drug resistant TB (MDR-TB) refers to TB in which TB bacteria isolated in vitro are resistant to at least isoniazid (INH) and rifampicin (RFP) at the same time (10). In recent years, there has been a lack of effective drugs for the treatment of drug-resistant TB, and the diagnosis and treatment of MDR TB are

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not optimistic. Related studies found that the resistance of rifampicin and isoniazid was related to the mutations of the patients' rpoB, katG, and inhA genes, which confirmed the molecular mechanism of TB resistance at the genetic level. It is helpful to establish a specific and sensitive detection method for the treatment of TB (11-14). The infection of pathogenic microorganisms is related to many factors, including exposure to pathogenic microorganisms, the virulence of pathogenic microorganisms, the body's immunity, and genetic factors. The MTB infection rate is very high, which accounts for about a third of the world's population, but only one in ten develop the disease. Its incidence is related to various factors, such as environmental factors, body resistance factors, and the degree of virulence of TB bacteria (15). The widespread prevalence of MDR-TB has brought severe challenges to public health in China. With the increase in population growth and population mobility, drug-resistant pulmonary TB cases in China are showing large quantification and diversification (16,17). The continuous spread of TB clinically resistant strains and high drug resistance rate has become the most difficult problem in TB prevention and control in China. The epidemic situation of drug-resistant TB is still quite severe, which should be paid great attention to by relevant departments.

To sum up, the prevention and treatment of TB/HIV dual infection still require a lot of research, and the internal mechanism of multidrug resistance genes in TB is not clear. Therefore, TB patients and HIV infected/AIDS patients registered in Fuzhou Center for Disease Control and Prevention were selected as research subjects in this study. To provide data support for the screening and prevention of TB/HIV dual infection, the population characteristics of TB/HIV dual infection and the mutations of drug-resistant genes were discussed.

Materials and methods

Research samples

TB patients and HIV-infected/AIDS patients registered in Fuzhou Center for Disease Control and Prevention from 2019 to 2020 were selected as the research subjects. This study had been approved by the medical ethics committee of the hospital. Patients and their families had been informed of this study and signed the informed consent for screening TB patients with HIV infection.

With the informed consent of the patients, blood was collected for HIV antibody testing, and the existing live HIV infected /AIDS patients were screened with a questionnaire for suspected symptoms of TB. Then, the screening questionnaire was filled out. Free chest radiographs and sputum smears were collected for HIV-infected /AIDS patients with suspected TB symptoms found by screening. Baseline data such as gender, age, and marital status were collected.

Two-way screening

According to the requirements of the *National HIV Testing Technical Specifications 2009 (Revised Edition)* (18), HIV testing was performed on TB patients. Whether or not patients were tested, they needed to be informed about the importance of preventing HIV infection, provided with knowledge about changing risk behaviors, preventing HIV infection and transmission, and information about prevention and treatment. Blood samples from HIV-positive TB patients in the initial screening and retest should be collected again for HIV confirmation test to increase the reliability of HIV antibody test results and avoid false positives. The HIV infection detection process was shown in Figure 1 below.

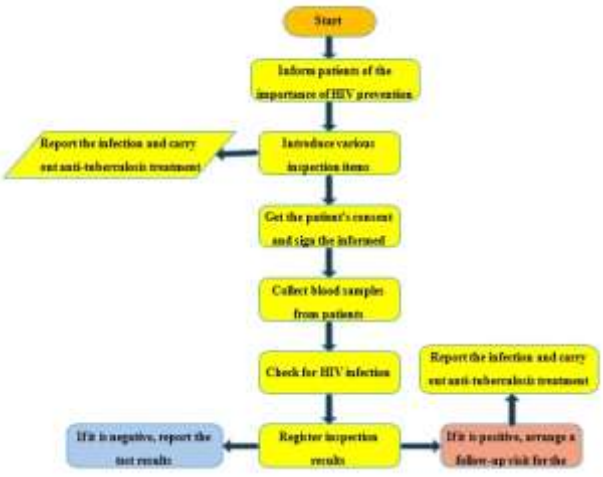

Figure 1. Flow chart of HIV infection detection

In accordance with the requirements of the *Guidelines for the Implementation of China's TB Prevention and Control Plan 2008 Edition* (19), HIV/AIDS patients were tested for TB, including

sputum smear and chest X-ray examination. Moreover, the register of newly diagnosed patients and the register of TB bacteriology experiments were filled in. For all confirmed sputum smear-positive and sputum smear-negative patients with active pulmonary TB, a medical record was established, and a TB patient registry should be filled in. In view of the characteristics of HIV combined with TB, it was necessary to combine the patient's medical history, clinical manifestations or characteristics, and laboratory pathology to make a comprehensive diagnosis for pulmonary TB with negative sputum smears and atypical lesions on chest radiographs. The diagnosis of extrapulmonary TB mainly depended on bacteriology and pathology. The TB inspection process was shown in Figure 2 below.

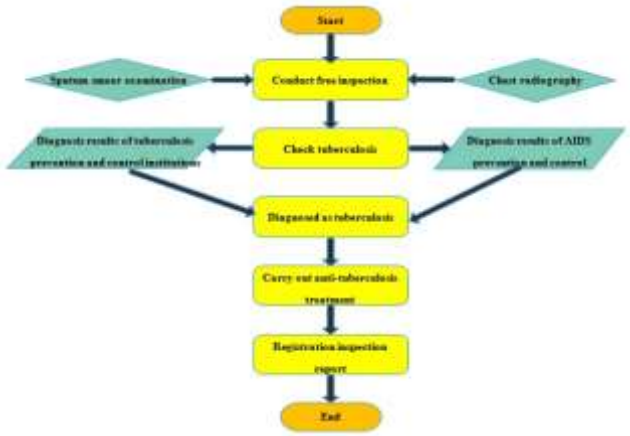

Figure 2. TB detection flow chart

Specimen collection and strain culture

Sputum specimen collection referred to collecting the morning sputum of TB patients, ensuring the aseptic operation, and storing the sputum in the refrigerator at 4°C or directly for testing.

The collection of specimens from other parts should be strictly aseptic when other body fluid specimens or tissue specimens of patients were collected, and the collection of urine specimens of patients should receive a full amount of nocturia.

MTB culture was as follows. Sputum specimen treatment: 4mL 2% NaOH was added to 2mL sputum sample, shaken and mixed for about 5-10 minutes. Centrifugation was performed at 12,000rpm for 10 min. Then, the supernatant was discarded, and the sample was washed with normal saline twice. 500μL normal saline was added and mixed well. Other specimens: urine, thoracic and abdominal fluid, cerebrospinal fluid, etc., were first left for a while, and then the precipitated part was centrifuged at 3,000r/min for 30 minutes. Then, the precipitated part was smeared. Tissue specimens were ground and smeared directly. Pus was treated as sputum. Firstly, 0.8mL of nutritional additives and bacteriostatic agent were added to the culture tube, and then 0.5mL of sediment was added. BACTEC MGIT960 automatic rapid mycobacterium culture and identification were used for detection.

The results were interpreted as follows. Ziehl-Neelsen acid-fast staining method was adopted for smear (20). The Mycobacteria growth indicator tube (MGIT) culture was mixed thoroughly, two drops (100μL) of the culture solution were dropped on a slide, and then it was painted into a rectangle (1×2 cm). The slides were then stained, soaked in carbolic magenta for about five minutes, and heated with a flame such as gas for five minutes until steam appeared. The second soak of carbolic fuchsin was made, and it was reheated with a gas flame. The surface of the slide was cleaned with distilled water for carbolic fuchsin, and then the slide was soaked in 3% acetic alcohol for about three minutes. The ethyl acetate was removed from the slides with distilled water, and the slides were immersed in methylene blue for about one minute. The methylene blue was off the slide with distilled water, which was dried and placed on a slide rack.

Drug sensitivity test

Experimental steps were as follows. I. Bacteriostatic agents and nutritional additives were added to all culture tubes and were labeled as the control, streptomycin, isoniazid, rifampicin, ethylambutol, amikacin, capreomycin, and ofloxacin, respectively. II. 0.1mL of streptomycin, isoniazid, refampicin, ethylambutol, amikacin, capreomycin, and ofloxacin reagent was added to each tube, 0.5mL of the original bacterial solution was added to each antimicrobial culture tube, and 0.5mL of 1:100 dilution of the original bacterial solution was added to the carer for two days. III. Then, 0.5mL 1:5 dilution of the original bacterial solution was added to the antibacterial culture tube, and 0.5mL 1:100 dilution of the original bacterial solution was added to the care, and the culture was five days. Finally, BACTEC

MGIT960 automatic rapid mycobacterium culture and identification were used for detection.

The interpretation of the results was as follows. I. Drug resistance: after being cultured for the specified time, the control tube was collected for two days, and then the drug-sensitive tube was collected. If the drug-sensitive tube was positive for TB, it was drug-resistant. II. Sensitivity: the control tube and the drug-sensitive tube were collected at the same time. If the control tube showed positive and the drug-sensitive tube did not appear positive, the drug was sensitive.

Extraction and amplification of bacterial genomic DNA

The cloned bacteria were scraped from Roche's medium 1 standard loop, which was added to the centrifuge tube in an 85°C water bath for 30 minutes, then boiled in boiling water for ten minutes, centrifuged at 12,000 r/min for 10 minutes. The supernatant was extracted and transferred to a new tube for storage at -20°C.

The rpoB, katG, and inhA gene sequences of the standard strain of MTB H37Rv were obtained from Genebank at http://ww.ncbi.nlm.nih.gov. Prime 5.0 was used for primer design.

GeneAlign in Lasergene was employed to compare the sequencing results with the gene sequence of the standard strain H37Rv to determine the gene mutation site.

Statistical methods

SPSS 19.0 was employed for data statistics and analysis. Mean ± standard deviation ($\overline{x}\pm s$) was how measurement data were expressed, and percentage (%) was how count data were expressed. One-way analysis of variance was used for pairwise comparison. The difference was statistically considerable with $P<0.05$.

Results and discussion

TB/HIV infection population characteristics

In Figure 3 below, a total of 2,990 TB samples were tested. There were certain differences in the characteristics of TB/HIV infection groups in different demographic variables. In terms of age, patients aged 20-40 had the highest HIV infection rate, followed by patients over 40 years old, and patients younger than 20 years old had the lowest HIV infection rate. In terms of gender, men were significantly more likely to be infected with HIV than women. In terms of marital status, the HIV infection rate of married patients was much lower than that of single patients. In terms of TB diagnosis, the HIV infection rate among extrapulmonary TB patients and smear-negative patients was significantly higher than that of smear-positive patients.

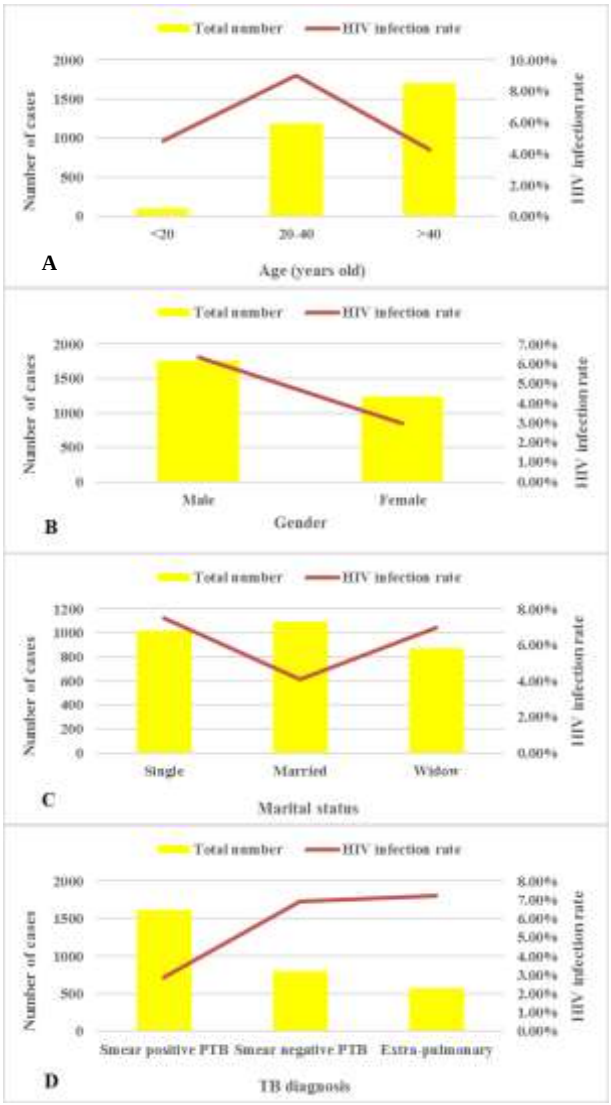

Figure 3. TB/HIV infection population characteristics. (A: the age of the subjects; B: the gender of the subjects; C: the marital status of the subjects; D: the diagnosis of the subjects' smear)

Population characteristics of TB infection in HIV infected/AIDS patients

In Figure 4 below, a total of 1,379 samples of HIV-infected/AIDS patients were tested. There were certain differences in the characteristics of the TB infection group of HIV-infected/AIDS patients in

different demographic variables. In terms of age, patients aged 20-40 had the highest TB infection rate, followed by patients over 40 years old, and patients younger than 20 years old had the lowest HIV infection rate. In terms of gender, men were significantly more likely to be infected with HIV than women. In terms of marital status, the HIV infection rate of married patients was much lower than that of single patients. In terms of TB diagnosis, the HIV infection rate among extrapulmonary TB patients and smear-negative patients was significantly higher than that of smear-positive patients.

Results of drug resistance and sensitivity of MTB to seven kinds of anti-TB drugs

Figure 5 showed the results of drug resistance and sensitivity of MTB to seven anti-TB drugs. Figure 5A showed that MTB had the highest resistance to isoniazid (42.86%), followed by ofloxacin (34.82%), streptomycin (33.81%), and rifampicin (32.15%), While the resistance to amikacin was the lowest (15.44%).

Further detailed analysis of the drug susceptibility of the initial treatment patients (Figure 5B) and retreatment patients (Figure 5C) samples was implemented. The resistance of MTB to the seven anti-TB drugs in the samples of newly treated patients was low (less than 30%), among which the resistance to isoniazid was the highest (24.72%), followed by ofloxacin (21.13%), capreomycin (19.11%), and streptomycin (18.41%). The MTB sample from the retreated patient was highly resistant to the seven kinds of anti-TB drugs. Among them, the resistance to isoniazid was the highest (73.27%), followed by rifampicin (65.44%) and streptomycin (62.81%).

The resistance of MTB to the combined application of first-line anti-TB drugs

Figure 6 showed the resistance results of MTB to the first-line anti-TB drugs (streptomycin, isoniazid, rifampicin, and ethambutol) in combination with two drugs. From Figure 6A, the total sample of MTB had the highest resistance to streptomycin + isoniazid (6.17%), followed by isoniazid + rifampicin (2.77%), and the resistance to streptomycin +Ethambutol was the lowest (0.34%). From Figure 6B, the MTB of the newly treated patient sample had the highest resistance to streptomycin + isoniazid (5.35%), followed by isoniazid + rifampicin (1.18%), and Streptomycin + ethambutol had the lowest resistance (0.49%). The MTB from the retreatment patient samples had the highest resistance to isoniazid + rifampicin (6.53%), followed by streptomycin + isoniazid (5.22%). The lowest resistance was to streptomycin + ethambutol (0.23%).

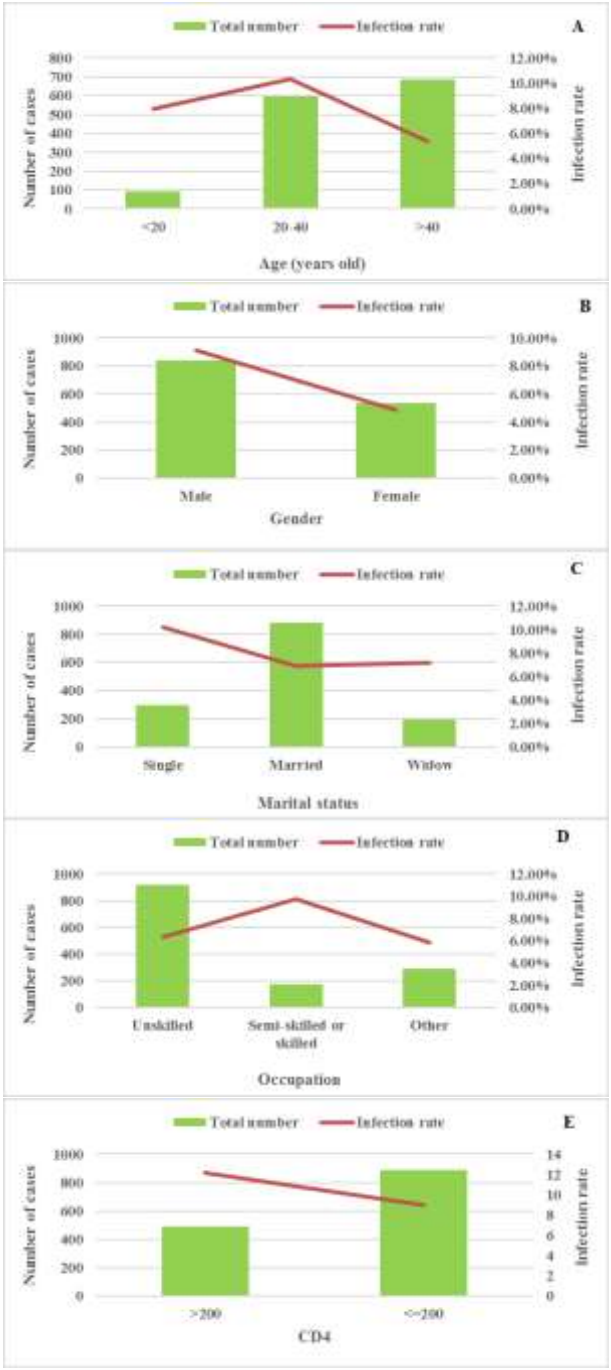

Figure 4. Population characteristics of TB infection among HIV infected/AIDS patients. (A: the patients' age; B: the patients' gender; C: the patients' marital status; D: the patients' occupation; E: the patients' CD4)

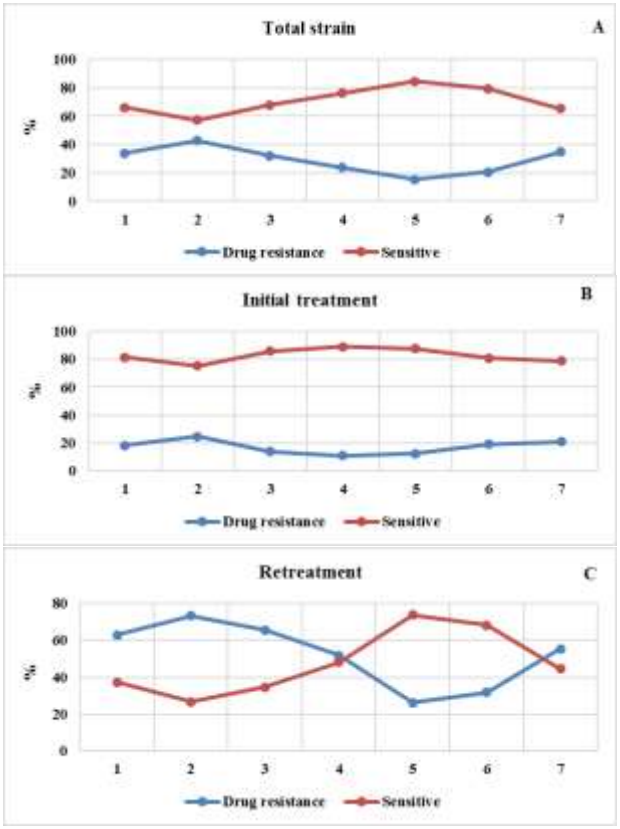

Figure 5. Results of drug susceptibility test. (A: the total sample resistance and sensitivity; B: the drug resistance and sensitivity of the initial treatment sample; C: the drug resistance and sensitivity of the retreatment sample). (1-7 indicated streptomycin, isoniazid, rifampicin, ethambutol, amikacin, capreomycin, and ofloxacin, respectively.)

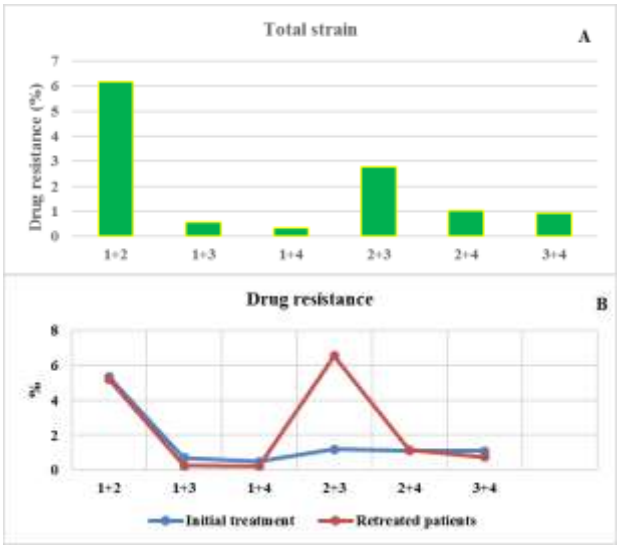

Figure 6. The drug resistance of MTB to the combined application of two drugs in the first-line anti-TB drugs. (A: the drug resistance of the total sample; B: the drug resistance of the initial treatment and retreatment samples; 1-4 indicated streptomycin, isoniazid, rifampicin, and ethambutol, respectively.)

Figure 7 showed the resistance results of MTB to the first-line anti-TB drugs (streptomycin, isoniazid, rifampicin, and ethambutol) in combination with three drugs. From Figure 7A, the total sample of MTB had the highest resistance to streptomycin + isoniazid + rifampicin (7.03%), followed by isoniazid + rifampicin + ethambutol (3.36%). The resistance to streptomycin + rifampicin + ethambutol was the lowest (0.58%). From Figure 7B, the MTB from the newly treated patient sample had the highest resistance to streptomycin + isoniazid + rifampicin (3.29%), followed by streptomycin + isoniazid + ethylamine Butanol (1.48%). The resistance to streptomycin + rifampicin + ethambutol was the lowest (0.35%). The MTB from the retreatment patient samples had the highest resistance to streptomycin + isoniazid + rifampicin (11.14%), followed by isoniazid + rifampicin + ethambutol (8.34%). The resistance to streptomycin + rifampicin + ethambutol was the lowest (1.54%).

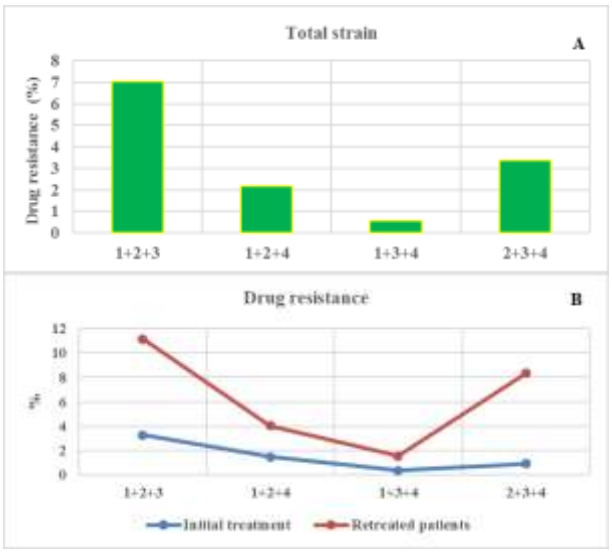

Figure 7. The drug resistance of MTB to the combined application of three drugs in the first-line anti-TB drugs. (A: the drug resistance of the total sample; B: the drug resistance of the initial treatment and retreatment samples; 1-4 indicated streptomycin, isoniazid, rifampicin, and ethambutol, respectively.)

Figure 8 showed the results of drug resistance of MTB to the first-line anti-TB drugs (streptomycin, isoniazid, rifampicin, and ethambutol) in combination with four drugs. The resistance of the total sample of MTB to streptomycin + isoniazid + rifampicin + ethambutol was 19.44%. The resistance of MTB to streptomycin + isoniazid + rifampicin + ethambutol in

samples from newly treated patients was 6.22%. The resistance of MTB to streptomycin + isoniazid + rifampicin + ethambutol in samples from retreatment patients was 38.25%.

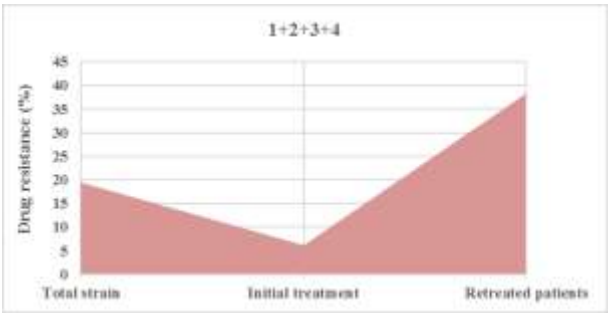

Figure 8. The resistance of MTB to the combined application of four drugs among the first-line anti-TB drugs. (1-4 indicated streptomycin, isoniazid, rifampicin, and ethambutol, respectively)

The resistance of MTB to the combined application of second-line anti-TB drugs

Figure 9 showed the resistance results of MTB to the combined application of two of the second-line anti-TB drugs (amikacin, capreomycin, and ofloxacin). From Figure 9A, the total sample of MTB had the highest resistance to amikacin + capreomycin (3.61%), followed by capreomycin + ofloxacin (1.99%). The resistance to amikacin + ofloxacin was the lowest (1.45%). From Figure 9B, MTB in the newly treated patient sample had the highest resistance to amikacin + capreomycin (4.05%), followed by amikacin + ofloxacin (1.56%). The resistance to capreomycin + ofloxacin was the lowest (0.72%). The resistance of MTB to capreomycin + ofloxacin in retreatment patient samples was the highest (5.31%), followed by amikacin + capreomycin (2.88%). The resistance to amikacin + ofloxacin was the lowest (2.54%) (Figure 10).

3.6 Gene mutation characteristics of multidrug-resistant MTB

In Figure 11 below, 113 cases of multidrug-resistant strains were finally detected from the results of drug susceptibility experiments. Among them, 82 cases had rpoB gene mutations, the gene mutation rate was 55.75%, and the mutation range was from 511 codons to 569 codons. There were 73 cases (89.02%) with mutations in the rifampicin resistance determining region (RRDR). The mutation frequency of codon 528 was the highest (54.08%), and the corresponding amino acid changes were Ser→Leu and Ser→Phe. The second was the mutation frequency of codon 523 (24.17%), and the corresponding amino acid changes were His→Asp and His→Tyr. The third was the 517 codon, the mutation frequency was 10.22%, and the corresponding amino acid changes were Asp→Gly and Asp→Tyr. The fourth was codon 562, the mutation frequency was 7.83%, and the corresponding amino acid change was Ile→Val. The fifth was codon 569, the mutation frequency was 3.11%, and the corresponding amino acid change was Asn→Ser. The sixth was the 511 codon, with a mutation frequency of 1.25%, and the corresponding amino acid change was Gln→Pro.

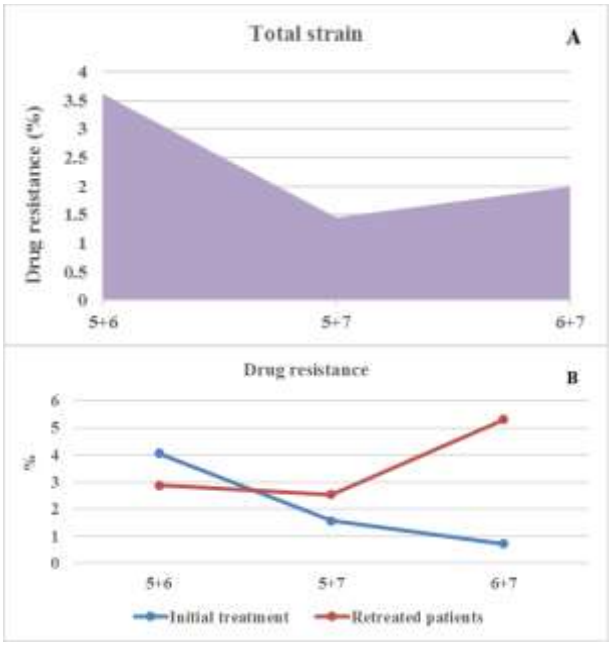

Figure 9. The resistance results of MTB to the combined application of two drugs in the second-line anti-TB drugs. (5-7 indicated amikacin, capreomycin, and ofloxacin, respectively.)

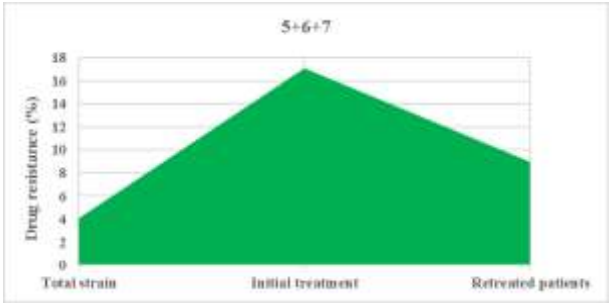

Figure 10. The resistance results of MTB to the combined application of three drugs in the second-line anti-TB drugs. (5-7 indicated amikacin, capreomycin, and ofloxacin, respectively.)

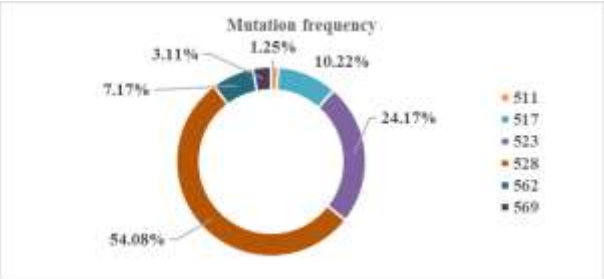

Figure 11. Mutation characteristics of rpoB gene of multidrug-resistant MTB

Figure 12 showed the mutation characteristics of the katG/inhA gene of multidrug-resistant MTB. Among the 113 cases of multidrug-resistant strains, 31 cases had mutations in the katG/inhA gene. Of which, there were 17 cases of katG single gene mutation, and the corresponding amino acid was changed to Ser315Thr. There were nine cases of inhA single gene mutation, and the corresponding amino acid changes were Ala5Ser and Ala5Cys. There were five cases of joint mutation of katG and inhA genes, and the corresponding amino acids changes were Val230Met and Pro232Gln.

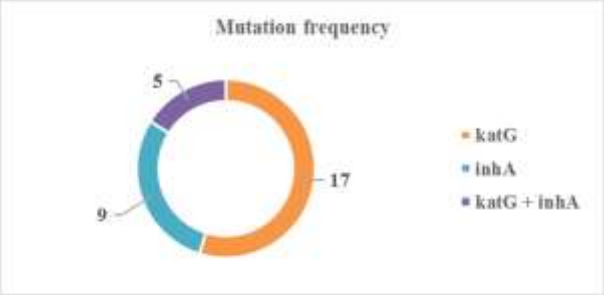

Figure 12. Mutation characteristics of katG/inhA gene of multidrug-resistant MTB

In recent years, with the increasing number of HIV infections, more and more HIV-infected people have entered the HIV onset stage. The immune function and CD4 cell count of HIV-infected persons decrease gradually, and the chance of dual infection increases, such as bacterial infection, fungal infection, and viral infection. Among them, TB bacilli infection is the most common and the fatality rate is the highest. TB/HIV dual infection has attracted great attention from the medical community (21,22). Although the transmission routes of TB and HIV are different, the relationship between them is very close, and it is easy to be infected with these two diseases at the same time or successively, leading to the dual infection of TB with HIV (23). In this study, TB patients and HIV infected /AIDS patients registered in Fuzhou Center for Disease Control and Prevention were selected as research subjects. The population characteristics of TB/HIV dual infection and mutation of drug-resistant genes were discussed. It was found that the population characteristics of TB/HIV infection were different in different demographic variables. In terms of age, patients aged 20-40 had the highest HIV infection rate, followed by patients aged over 40, and patients aged younger than 20 had the lowest HIV infection rate, which was similar to the study results of Teixeira et al. (2018) (24). The reason may be that people over 40 were sexually active and had higher rates of risky sex. HIV infection rate in patients with extrapulmonary TB and smear-negative patients was significantly higher than that in smear-positive patients, which was different from the study results of Hayibor et al. (2020) (25). This may be because TB patients with negative sputum smears tended to ignore their TB infection status and had poor awareness of prevention and control of various risky behaviors. HIV screening should therefore be given priority in patients over 40 years of age with smear-negative TB/extrapulmonary TB. By analyzing the characteristics of the TB infection group in HIV /AIDS patients, it was found that the TB infection rate was the highest in 20-40 years old patients, and the probability of HIV infection in men was significantly higher than that in women. In addition, the prevalence of HIV in married patients was significantly lower than in single patients, suggesting the need to focus on screening for men, single, and people aged 20-40 with HIV /AIDS.

Further, the drug-resistant genes were extracted and identified. It was found that the resistance of MTB to isoniazid was the highest (42.86%), followed by ofloxacin (34.82%), streptomycin (33.81%), and rifampicin (32.15%). However, it had the lowest resistance to amikacin (15.44%). The MTB resistance of the re-treated patients to the seven kinds of anti-TB drugs was higher than that of the newly treated patients. The results showed that MTB had high resistance to anti-TB drugs, posing a great threat and challenge to the prevention and treatment of TB in this region (26). From the results of drug susceptibility experiments, 113 cases of multidrug-resistant strains were finally detected, of which 82

cases had rpoB gene mutations, and the gene mutation rate was 55.75%. The mutation ranged from codon 511 to codon 569, and 73 cases (89.02%) had mutations in the rifampicin resistance determining region (RRDR), which suggested that this region was the main mutation location that led to rifampicin resistance (27). In addition, the mutation frequency of codon 528 was the highest (54.08%), and the corresponding amino acid changes were Ser→Leu and Ser→Phe, which indicated that the mutation at this codon site was polymorphic. Among the 113 cases of multidrug-resistant strains, 31 cases had mutations in the katG/inhA gene. Of which, there were 17 cases of katG single gene mutation, and the corresponding amino acid change was Ser315Thr, which meant that the katG Ser315Thr mutation was a very common and representative mutation form.

Conclusions

In this study, TB patients and HIV infected /AIDS patients registered in Fuzhou Center for Disease Control and Prevention were selected as research subjects. The population characteristics of TB/HIV dual infection and mutation of drug-resistant genes were discussed. The results showed that TB/HIV bidirectional screening was necessary for patients over 40 years of age with smear-negative TB and for men, single, and HIV /AIDS patients between 20 and 40 years of age. In addition, the resistance of MTB to anti TB drugs in this area was generally high, and the drug resistance of retreated patients was significantly higher than that of newly treated patients. RpoB mutations were found in 82 of 113 multidrug-resistant strains, with a mutation rate of 55.75%, ranging from codon 511 to codon 569. Thirty-one cases had katG/inhA gene mutations. Among them, there were 17 cases of katG single-gene mutations, 9 cases of inhA single-gene mutations, and 5 cases of katG and inhA gene mutations. However, this study may have potential biases in the selection of research subjects, and there are fewer indicators of demographic variables. It is necessary to further discuss indicators such as smoking, drinking, drug use, and commercial sex in the future. In conclusion, this study provides data support for the screening and prevention of TB/HIV dual infection.

Conflict interest

The authors declare that they have no conflict of interest.

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